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(54) Process for producing N-chloroacetylglutamine.

(57) N-chloroacetylglutamine is produced by reacting chloroacetyl chloride with an alkaline aqueous solution of glutamine in the presence of a water-immiscible organic solvent, separating an aqueous layer by liquid-liquid separation, and crystallizing N-chloroacetyl-glutamine from the aqueous layer under acidic conditions. N-Chloroacetylglutamine useful as an intermediate for producing glycyl-L-glutamine which has higher stability than L-glutamine and is used as a component of an infusion solution can be obtained with high efficiency at low cost.

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aqueous solution containing an organic base, such as trimethylamine, triethylamine or pyridine. Sodium hydroxide, potassium hydroxide, sodium carbonate and potassium carbonate are preferred alkalis.

The concentration of the glutamine to be employed in the aqueous alkaline solution may be from 0.01 to 3 M, preferably from 0.1 to 1 M.

The water-immiscible organic solvents include ether, toluene, chloroform, methylene chloride, dichloroethane, ethyl acetate, and mixtures thereof. Toluene, chloroform, and methylene chloride are preferred.

While not limiting, the organic solvent is used in an amount 0.1 to 5, preferably 0.3 to 1, times the volume of the alkaline aqueous solution of glutamine.

Since hydrochloric acid is formed with the progress of the reaction which reduces the pH of the reaction mixture, the reaction mixture is adjusted to a pH between 7 and 13, preferably between 10 and 12, during the reaction.

Glutamine is used in an amount usually of from 0.5 to 2.0 equivalents, preferably 1 equivalent, to chloroacetyl chloride.

The reaction is carried out at a temperature of -5° to 40°C, preferably 0° to 10°C, for a period of 0.1 to 5 hours, preferably 0.5 to 2 hours.

In general, an acid halide is apt to be hydrolyzed and inactivated in an aqueous alkaline solution. In the present invention, since the acid halide exists in a water-immiscible organic solvent, it hardly undergoes any decomposition with an alkali, whereby the desired reaction proceeds on an interface between an aqueous alkaline solution layer and an organic solvent layer to achieve a high yield.

After completion of the reaction, the organic solvent is removed from the reaction mixture by liquid-liquid separation. The aqueous alkaline solution layer thus separated is adjusted to a pH of 0.1 to 3, preferably 1 to 2, with a strong acid, such as hydrochloric acid or sulfuric acid, and, after seeding, cooled to obtain crystals of crude N-chloroacetylglutamine in good yield.

Recrystallization of the resulting crude N-chloroacetylglutamine from water gives purified N-chloroacetylglutamine. Alternatively, the crude N-chloroacetylglutamine is suspended in an appropriate organic solvent or a mixed solvent of an organic solvent and water, the resulting suspension is stirred, an insoluble salt is removed by filtration, and the filtrate is subjected to recrystallization to obtain purified N-chloroacetylglutamine.

The organic solvent which can be used in the above-described purification procedure includes alcohols, such as methanol, ethanol and propanol, and acetone, with ethanol being preferred. The solvent is used in an amount of 0.5 to 10 times, preferably 1 to 5 times, the weight of the crude N-chloroacetylglutamine.The stirring and filtration are performed at a temperature of 5 to 50°C, preferably 30 to 40°C. The stirring is continued for 5 minutes to 3 hours, preferably 10 minutes to 1 hour.

If desired, the resulting pure N-chloroacetylglutamine can further be recrystallized from water to achieve higher purity.

The crude N-chloroacetylglutamine as referred to above may be reacted, without being purified, with an aqueous solution containing ammonia to afford glycylglutamine with high purity in high yield.

The present invention provides a process for producing N-chloroacetylglutamine useful as an intermediate for producing glycylglutamine, with high purity at low cost from the industrial viewpoint, in which N-chloroacetylglutamine produced can be isolated and purified efficiently without complicated extraction.

The present invention will now be illustrated in greater detail with reference to Examples and Reference Example, but it should be understood that the present invention is not deemed to be limited thereto.

EXAMPLE 1

Synthesis of N-Chloroacetyl-L-glutamine

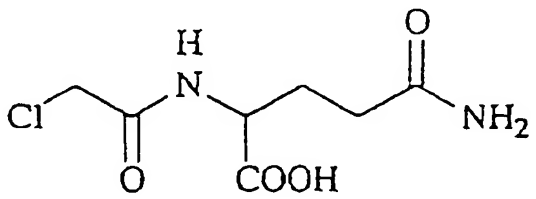
To a mixed solvent of 1750 ml of water and 497 ml of toluene was added 733.7 g (5 mol) of L-glutamine at room temperature. The solution was cooled to 0 to 5°C and adjusted to pH 11 with 5N sodium hydroxide. To the solution was added dropwise a solution of 565.3 g (5 mol) of chloroacetyl chloride in 497 ml of toluene while maintaining the pH of the reaction mixture at 11 with 5N sodium hydroxide. After stirring at 0 to 5°C for 1 hour, toluene was removed from the reaction mixture by liquid-liquid separation. The aqueous layer separated was adjusted to pH 2 by addition of 410 ml of concentrated hydrochloric acid. After seeding, the reaction mixture was subjected to crystallization at room temperature for 1 hour and then at 0 to 5°C for 3 hours. The resulting crystals were collected by filtration and dried under reduced pressure to give 992.6 g (89.2 mole percent (mol%) yield) of crude N-chloroacetyl-L-glutamine (sodium chloride content: 13.6 weight percent (wt%)). The crystals were analyzed by high performance liquid chromatography (HPLC) (column: Shim-pack CLC-ODS, 6 x 150 mm (manufactured by Shimadzu Corporation); eluent:

form a solution. The solution was cooled to room temperature, and 386.0 g (1.5 mol) of crude N-chloroacetyl-L-glutamine as obtained in Example 1 was added thereto, followed by allowing the mixture to react at 40 °C for 8 hours. The reaction mixture was concentrated under reduced pressure, and 2050 ml of water was added to the residue, followed by concentration again. To the residue was added 650 ml of water to make the total weight 1162 g. Ten grams of activated carbon were added thereto, and the mixture was stirred at 50 °C for 30 minutes. The activated carbon was washed with 166 ml of water and filtered while hot. To the filtrate was added dropwise 550 ml of methanol at 50 °C. After seeding, 550 ml of methanol was further added, followed by cooling to 0 to 5 °C, at which crystallization was continued for 3 hours. The resulting crystals were collected by filtration and dried under reduced pressure to obtain 250.8 g (75.6 mol% yield) of glycyl-L-glutamine. As a result of HPLC under the same conditions as in Example 1, the purity of the resulting product as calculated from the HPLC relative area ratio was 97.2%.

While the invention has been described in detail and with reference to specific examples thereof, it will be apparent to one skilled in the art that various changes and modifications can be made therein without departing from the spirit and scope thereof.

Claims

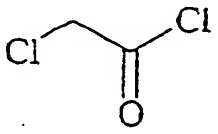
1. A process for producing N-chloroacetylglutamine represented by formula (I):



(I)

comprising the steps of:

(a) reacting chloroacetyl chloride represented by formula (II):



(II)

with an alkaline aqueous solution of glutamine in the presence of a water-immiscible organic solvent;

(b) separating an aqueous layer by liquid-liquid separation; and

(c) crystallizing N-chloroacetylglutamine from said aqueous layer under acidic conditions.

2. The process of claim 1, wherein in step (a) the pH is maintained between 7 and 13.

3. The process of claims 1 or 2, wherein the water-immiscible solvent is selected from ether, toluene, chloroform, methylene chloride, dichloroethane, ethyl acetate and mixtures thereof.

European Patent Office

EUROPEAN SEARCH REPORT

Application Number
EP 95 10 5656

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.6)
A,D	HOPPE-SEYLER'S ZEITSCHRIFT FÜR PHYSIOLOGISCHE CHEMIE, vol. 105, 1919 BERLIN - LEIPZIG, pages 58-82, H. THIERFELDER ET AL. 'Über glutaminhaltige Polypeptide und zur Frage ihres Vorkommens in Eiweiss' * page 62 - page 65 *	1-3	C07C231/10 C07C237/22
A,D	JOURNAL OF THE NATIONAL CANCER INSTITUTE, vol. 7, no. 4, February 1947 pages 275-279, VINCENT E. PRICE ET AL. 'Studies on the effect of pyruvate on the desamidation of glutamine, asparagine, and related compounds' * page 275 *	1-3	
A	US-A-3 206 506 (HAROLD W. GRIFFITH ET AL.) * claims 1-15; examples 1,2 *	1-3	
A,D	DATABASE WPI Section Ch, Week 9502 Derwent Publications Ltd., London, GB; Class B05, AN 88-101702 & JP-A-63 051 399 (AJINOMOTO KK) , 4 March 1988 * abstract *	1	TECHNICAL FIELDS SEARCHED (Int.Cl.6) C07C
A,D	AUSTRALIAN JOURNAL OF CHEMISTRY, vol. 7, no. 2, May 1954 pages 173-180, S. J. LEACH ET AL. 'The preparation of some dipeptides containing asparagine, aspartic acid, and glutamine' * the whole document *	1	

The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
'BERLIN	23 June 1995	Rufet, J

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
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